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Poster session 1: Oncology, renal physiology and disease, neurology, infectious diseases, new topics of endothelin biology

The role of endothelin-1 in the vascular pathobiology of cerebral malaria

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Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection associated with cerebral vasculopathy, high mortality, and risk of neurological sequelae. In human CM, infected RBCs adhere to the brain endothelium and occlude the cerebral blood vessels causing cerebral vascular damage, impaired perfusion, vasospasms, vasoconstriction, and inflammation. Vasoactive factors, including endothelin (ET-1), have become increasingly important in the pathogenesis of CM. We previously demonstrated that antagonism of the ET-1 type A receptor (ETA) improved survival and attenuated brain hemorrhage in murine CM. In this study we tested the hypothesis that ET-1 contributes to CNS inflammation and BBB disruption in experimental CM (ECM) via its actions on ETA. To test this hypothesis we used our model of *Plasmodium berghei* ANKA (PbA) infection of C57BL/6 mice. PbA-infection resulted in activation of monocytic CNS cells, microglia, which are important in inflammation. ECM was also associated with an increase in brain microvascular endothelial cell activation which is critical for leukocyte adhesion. Treatment of PbA-infected mice with ETA receptor antagonists attenuated the increase in microglial and endothelial cell activation, suggesting that ET-1 contributes to CNS inflammation during ECM. Furthermore, leakage of Evans blue bound-albumin into the brain was reduced in ECM mice receiving ETA receptor antagonism, providing further support that disruption of the BBB and inflammation during ECM result, in part, from increases in ET-1 and its actions on the ETA receptor. Together these findings illustrate a role for ET-1 in the immunopathology and vasculopathy associated with ECM, and highlight the peptide as a potential target for adjunctive therapy for the protection of neurological function in patients with CM.

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Dual endothelin blockade exacerbates upregulated VEGF angiogenic signaling in the heart of a lipopolysaccharide-induced endotoxemic rat model

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Sepsis, a heterogeneous class of syndromes, is associated with the development of progressive damage in multiple organs. The pathogenesis of sepsis-induced myocardial dysfunction is still not fully understood. The present study examined the alteration of key angiogenic signaling pathway mediated by the vascular endothelial growth factor (VEGF) in sepsis heart and the effects of dual endothelin (ET) antagonism on it. Normal male Wistar rats at age 8 wks were administered with lipopolysaccharide (LPS: 15 mg/kg) and then sacrificed at different time points (1 h, 3 h, 6 h and 10 h). Some rats without LPS administration was considered as the control group. Some of the LPS-administered rats were treated with dual endothelin blocker (SB209670, 1 mg/kg body weight) for 6 h and then sacrificed. Administration of LPS resulted in increases in the serum levels of TNF-alpha (maximum at 1 h after LPS, 1200-fold compared to control rats), and ET-1 (maximum at 3 h after LPS, 25-fold compared to control rats). At 6 h after LPS administration, we found decreased percent of fractional shortening in the heart. The expression of VEGF, and its downstream angiogenic signaling molecules namely eNOS and NO, was significantly increased in heart tissues after LPS administration compared to the control group which was also accompanied by increased cardiac ET-1 level. Dual endothelin blockade for 6 h further upregulated the VEGF angiogenic signaling in endotoxemic heart.

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Endothelin plasma and tissue expression in ductal carcinoma of the breast: Correlation with clinicopathological characteristics and VEGF

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Purpose: Endothelin-1 (ET-1) is overexpressed in breast carcinomas, while circulating levels of its precursor (Big ET-1) have also been found elevated. In the present study, we evaluated plasma ET-1

and Big ET-1 levels, and tissue expression of ET-1 in patients with ductal carcinoma of the breast. Methods: Peripheral venous blood samples were collected prior to diagnostic biopsy from women with suspicious non-palpable mammographic lesions. Plasma ET-1 and Big ET-1 levels were determined in 30 patients with IDC, 30 with DCIS and 30 with benign lesions (controls), by performing ELISA. ET-1 and VEGF tissue expression was immunohistochemically determined. Potential correlations with histological grade, hormone receptor status, Her2/neu amplification, tumor size, lymph node involvement and disease stage were investigated in IDC. Results: Big ET-1 plasma levels were significantly higher in IDC and DCIS patients compared to controls ($p < 0.001$ and $p < 0.01$, respectively). No significant differences in ET-1 levels were observed between the three groups. Moderate to strong IHC staining for ET-1 was observed in 3/29 and 7/23 IDC and DCIS patients, respectively. VEGF was significantly expressed in 8/27 and 8/23 IDC and DCIS patients, respectively. In IDC, plasma and tissue expression of ET-1 and plasma expression of Big ET-1 did not correlate with any of the analyzed clinicopathological characteristics or VEGF tissue expression. Conclusions: Plasma levels of Big ET-1 were a more sensitive indicator of ET-1 deregulation than those of ET-1 in our study. Our results support the potential clinical application of Big ET-1 as a breast cancer biomarker.

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The localisation and distribution of endothelin receptors in normal and cancer colon tissues: Confirmation by autoradiography, immunohistochemistry and quantum dot targeting

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Background: Endothelin-1 (ET-1) acts via two G-protein-coupled receptors, ETA and ETB. Overexpressed ET-1 and ETA in colorectal cancer (CRC) promote tumour growth and progression. Aim: To investigate (1) ETA and ETB distribution in normal and cancer tissues from patients with CRC and (2) determine ETA and ETB localisation to cell types and tissue structures. Methods: ETA and ETB distribution was determined using in vitro autoradiography with competitive inhibition, using receptor antagonists (BQ123, ZD4054, BQ788) on normal and cancer tissues resected from patients with CRC ($N = 8$). Immunohistochemistry (IHC) confirmed ETA and ETB expression and identified associated cells/structures. ETA distribution was also investigated by quantum dots (QDs) conjugated to BQ123 (ETA-antagonist). Results: In normal bowel epithelium, ETA was observed closer to the luminal surface and ETB towards the muscularis mucosa/lamina propria. There was greater ETA than ETB binding in CRC. Both cancer and normal tissues demonstrated strongest binding to stromal cells, particularly fibroblasts (IHC). QD-BQ123 demonstrated an ETA punctate pattern in stromal areas surrounding epithelial cells; and an ETA increase in CRC compared to normal. Conclusions: ET-1 binds strongly to CRC stromal structures, with ETA greater than ETB, and is consistent with ET-1 signalling contributing to tumourigenesis. Within normal tissue, differential ETA and ETB distribution (luminal versus muscularis mucosa/lamina propria) has not been reported previously. This may relate to trophic, growth arrest and differentiation signalling. This study demonstrates the effective, novel use of receptor-antagonist-conjugated QDs; reveals possible ET-1 roles in normal tissue; and provides further evidence for the potential therapeutic use of ETA antagonists as CRC adjuvant treatment.

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Novel molecular pathways by which ETA receptor mediates tumourigenic signals in colorectal cancer: Support for ETA receptor antagonism as adjuvant treatment

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Background: The endothelin A receptor (ETA) mediates tumourigenic signals in colorectal cancer (CRC). The ETA ligand, endothelin-1 (ET-1), stimulates not only cancer cells but also surrounding fibroblasts and may promote the creation of a supporting tumour stroma. Aim: To identify ET-1 regulated genes associated with oncogenic pathways in colonic fibroblasts. Methods: Micro-array analysis following 4 h ET-1 stimulation of colonic fibroblast strains (isolated from patients undergoing resection for CRC, $n = 4$) identified differentially expressed genes ($n = 19$) at significant levels. Three were investigated further: COLXI, AML-1, and EGFR (collagen type-XI; acute myeloid leukemia-1; epidermal growth-factor receptor). Quantitative RT-PCR (qRT-PCR) and immunoblotting evaluated AML-1 and COLX expression levels, following treatment with ET-1 and/or receptor antagonists (ETA: BQ123, ZD4054; ETB: BQ788). ETA and ETB regulation of EGFR was investigated by gene silencing (siRNA); these assays and ET-1 regulation of EGFR over 24 h were evaluated by qRT-PCR. Results: ET-1 stimulated expression of AML-1 and COLXI at both gene (>1.5-fold; $p < 0.01$) and protein ($p < 0.05$) levels; stimulation was inhibited by ETA, but not by ETB, antagonism (AML-1: 75.1–77.1% by BQ123, ZD4054; COLXI: 65.1% by ZD4054; $p < 0.05$). EGFR expression demonstrated a biphasic increase at 4 h and 24 h (3.8-fold; 4.5-fold). Silencing ETA, but not ETB, returned EGFR levels to control. Conclusions: ETA antagonism has potential for targeting oncogenic pathways: AML-1 is linked to c-Jun N-terminal kinase which inhibits apoptosis/promotes proliferation; and abnormal TGF-β (transforming growth-factor-beta) signalling. COLXI is linked to CRC tumourogenesis. The ET-1-stimulated biphasic EGFR response and ETA antagonism have not been reported before in CRC. These findings identify mechanisms by which ETA promotes tumourigenesis and support addition of ZD4054 to existing EGFR antagonism therapy.

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Serum big endothelin-1 as a clinical marker in canine pulmonary hypertension and tumors

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